monolayer in standard conditions. Gene expression was assessed using real time RT-PCR. Sulfated proteoglycan content of micromass cultured chondrocytes was measured using Alcian blue staining followed by guanidine extraction and spectrophotometric quantification. TRPC6 was specifically activated using hyp9, a stabilized derivative of the TRPC6-selective activator hyperforin. Intracellular calcium chelation was achieved by treating cells with BAPTA-AM. AKT phosphorylation was analyzed using Western blot. Calcium mobilization in chondrocytes was measured using a fura-2 calcium influx assay.
Results: TRPC6 mRNA was detected in wild type murine chondrocytes. Culture of chondrocytes from TRPC6-/- and CXCR2-/- mice in micromass resulted in significantly less sulfated proteoglycan production in comparison to chondrocytes obtained from wild type littermates. Chondrocytes lacking either TRPC6 or CXCR2 expressed lower levels of the chondrocyte differentiation markers SOX9 and type II collagen in comparison to wild type. Activation of CXCR2 in articular chondrocytes in vitro resulted in an increase in intracellular calcium mobilization. In vitro activation of TRPC6 using hyp9 led to an increase in AKT phosphorylation in wild type chondrocytes, whereas chelation of intracellular calcium inhibited CXCL6 induced phosphorylation of AKT. Finally, activation of TRPC6 in wild type chondrocytes resulted in a significant increase in SOX9 and type II collagen mRNA expression.
Conclusions: CXCR2 signaling and in particular TRPC6 ion channel activity is required for chondrocyte phenotypic stability in vitro. TRPC6 mediated calcium mobilization is sufficient to increase AKT phosphorylation and the expression of key chondrocyte molecular markers in murine chondrocytes, indicating that TRPC6 may be an ideal therapeutic target in the prevention of articular cartilage breakdown during osteoarthritis.

226
THE ACUTE RESPONSE TO JOINT DESTABILISATION IS DIFFERENTIALLY REGULATED BETWEEN MALE AND FEMALE MICE

C. Driscoll †, F.E. Watt †, F. Dell'Accio ‡, T.L. Vincent †. † *The Kennedy Inst., Oxford, United Kingdom;* ‡ *William Harvey Inst., Queen Mary, University of London, United Kingdom*

Purpose: Gender is known to influence the severity of osteoarthritis in mice, with female mice having less cartilage degradation compared with males. We tested 3 separate hypotheses to account for this difference: (i) that physical activity levels are lower in female mice, (ii) that female mice are able to repair cartilage more effectively than male mice (iii) that female mice respond to acute joint injury (destabilization) with a different molecular profile. We validated the latter in a cohort of those with acute destabilising knee injuries.
Methods: The following measures were carried out in 10 week old male and female mice following destabilization of the medial meniscus (DMM): Activity levels were measured using LABORAS (laboratory Animal Behaviour Observation, Registration and Analysis System) 2 weeks following surgery. This allows quantification (over a 6h period) of animal behaviours such as running, climbing, feeding and grooming. For gene expression studies, mRNA was extracted from whole joints 6h following surgery and RT-PCR performed for 48 previously validated genes using Taqman microfluidic cards. For investigation of cartilage repair, 10 week old male and female C57Bl6 mice underwent focal cartilage injury in the patella groove as previously described (Eltawil et al, 2009). Joints were scored at 8 weeks for evidence of intrinsic repair. Human synovial fluid from male and female patients within 8 weeks of acute knee injury was analysed for selective proteins by Mesoscale Discovery platform or by plate ELISA.
Results: Activity levels between male and female mice were not significantly different (although there was a trend for female mice to be more active than male mice). In C57 Bl6 mice, there was no evidence of spontaneous repair of focal full thickness cartilage lesions, irrespective of gender. When examining the response to acute joint destabilization there was no difference in the regulation of many inflammatory response genes such as proteases (Adamts4, Adamts5), inflammatory cytokines (IL1b, IL6), chemokines (Ccl2, Ccl7), but 4 genes were consistently higher in female versus male joints. These were Timp1, activin bA, versican and Mmp3. Of the genes tested in human synovial fluid samples, activin bA protein levels were significantly higher in female patients compared with male patients post injury.
Conclusions: These results suggest that the initial injury response in female mice and, to some extent, in humans is different to the response in males. Genes that were significantly higher in female mice included Timp1 and activin bA (a TGFb family member), which are strongly FGF2-dependent genes in vivo and have predicted chondroprotective roles. The mechanism by which these genes may be protective is being investigated.

227
SYNDECAN-4 REGULATES CHONDROCYTE EXTRACELLULAR MATRIX ARCHITECTURE AND MODULATES WNT3A-INDUCED MATRIX REMODELING

C.K. Clarke †, U. Hansen †, R. Stange †, F. Echtermeyer ‡, F. Dell'Accio §, T. Pap †, J. Bertrand †. † *Inst. of Experimental Musculoskeletal Med., Muenster, Germany;* ‡ *Translational Perioperative Inflammation Res., Med. Sch. Hannover, Hannover, Germany;* § *William Harvey Res. Inst., Ctr. for Experimental Med. and Rheumatology, London, United Kingdom*

Purpose: Syndecan-4 (Sdc4), a family member of type I transmembrane heparan sulfate proteoglycans (HSPGs), is involved in various cartilage-related processes including osteoarthritis (OA). Blockade of Sdc4 signaling protects mice from cartilage degradation in experimentally induced OA. OA is characterized by hypertrophic differentiation of chondrocytes and extracellular matrix (ECM) remodeling, which may be at least partially triggered by the WNT signaling pathway. Our experiments emphasize the cross-talk of Sdc4 and WNT signaling and its influence on chondrocyte matrix production and remodeling.
Methods: In vitro analyses were performed using neonatal wild type (wt) and Sdc4-/- chondrocytes. The influence of WNT3a on glycosaminoglycan (GAG) production was analyzed using alcian blue staining of micromass cultures. Expression of marker genes (e.g. aggrecan, collagen2, MMP13) was measured by quantitative RT-PCR. Fibril structure and remodeling of the matrix upon WNT3a stimulation (with high concentrations resembling activation of canonical and low concentrations promoting non-canonical WNT signaling) was monitored by electron microscopy using micromass cultures. In vivo relevance was investigated upon induction of OA using the DMM model.
Results: Micromass cultures revealed a higher basal GAG production by Sdc4-/- chondrocytes. WNT3a stimulation led to a decrease in GAG production in wt cells, which was absent in Sdc4-/- chondrocytes. qRT-PCR showed a 10x higher basal production of aggrecan and collagen2 in Sdc4-/- chondrocytes. WNT3a increased the expression of both genes in Sdc-4-/-, whereas it decreased the expression in wt chondrocytes. MMP13 was significantly less expressed in Sdc4-/- chondrocytes and, unlike in wt cells, was not upregulated upon WNT3a stimulation.
Sdc4-/- cartilage displayed finer, more interspaced and less-orientated fibril structures compared to wt cartilage. Using micromasses, in wt we found an increase in fibril production with canonical and on the other hand a decrease in non-canonical WNT signaling, whereas in Sdc4-/- micromasses, the fibril production was hardly influenced. Furthermore, our results indicate that non-canonical WNT signaling induces fibril orientation. In vivo studies confirmed in vitro results.
Conclusions: Sdc4 influences fibril architecture, condensation and orientation as well as proteoglycan production of the chondrocyte ECM. Furthermore, Sdc4 cross-talks with the canonical WNT signaling pathway in regulating matrix remodeling.
We hypothesize that the restructured matrix in Sdc4-deficient cartilage contributes towards the protection from OA-induced changes by modulating the response to WNT signaling.

228
HYPOXIA-INDUCIBLE FACTOR 3-ALPHA EXPRESSION (HIF-3α) IS ASSOCIATED WITH THE STABLE CHONDROCYTE PHENOTYPE AND INHIBITS HIF-2α/ARNTL-MEDIATED TRANSACTIVATION OF MATRIX METALLOPROTEINASE-13

B.D. Markway †, H. Cho †, D.E. Anderson †, J. Zilberman-Rudenko †, P. Holden †, A. McAlinden ‡, M. Otero §, M.B. Goldring §, B. Johnstone †. † *Oregon Hlth.& Sci. Univ., Portland, OR, USA;* ‡ *Washington Univ., St. Louis, MO, USA;* § *Hosp. for Special Surgery and Weill Cornell Med. Coll., New York, NY, USA*

Purpose: Hypoxia-inducible factor 2α (HIF-2α) is a potent transactivator of the hypertrophy-related genes, MMP13, COL10A1, and VEGFA; particularly when dimerized with the β-subunit, aryl hydrocarbon receptor nuclear translocator-like (ARNTL). However, when redifferentiating chondrocytes are subjected to a hypoxic environment wherein HIF-2α is stabilized, we do not observe upregulation of MMP13